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5 METHODS AND COMPOSITIONS RELATING TO CD39-LIKE POLYPEPTIDES
AND NUCLEIC ACIDS

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1. FIELD OF THE INVENTION

The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with therapeutic, diagnostic and research utilities for these polynucleotides and proteins.

2. BACKGROUND OF THE INVENTION

Technology aimed at the discovery of protein factors (including e.g., cytokines, such as lymphokines, interferons, CSFs and interleukins) has matured rapidly over the past decade. The now routine hybridization cloning and expression cloning techniques clone novel polynucleotides "directly" in the sense that they rely on information directly related to the discovered protein (i.e., partial DNA/amino acid sequence of the protein in the case of hybridization cloning; activity of the protein in the case of expression cloning). In addition, more recently, "indirect" cloning techniques have been utilized, such as signal sequence cloning, which isolates DNA sequences based on the presence of a now well-recognized secretory leader sequence motif, as well as various PCR-based or low stringency hybridization cloning techniques, coupled in certain instances with database searching, have advanced the state of the art by making available large numbers of DNA/amino acid sequences for proteins that are known to have biological activity by virtue of their secreted nature in the case of leader sequence cloning, by virtue of the cell or tissue source in the case of PCR-based techniques and/or by virtue of their sequence similarity via database searches. It is to these proteins and the polynucleotides encoding them that the present invention is directed.

3. SUMMARY OF THE INVENTION

The compositions of the present invention include novel isolated polypeptides, in particular, novel CD-39-like polypeptides, isolated polynucleotides encoding such
5 polypeptides, including recombinant DNA molecules, cloned genes or degenerate variants thereof, especially naturally occurring variants such as allelic variants, and antibodies that specifically recognize one or more epitopes present on such polypeptides.

The compositions of the present invention additionally
10 include vectors, including expression vectors, containing the polynucleotides of the invention, cells genetically engineered to contain such polynucleotides and cells genetically engineered to express such polynucleotides.

The isolated polynucleotides of the invention include, but are not limited to, a polynucleotide encoding a
15 polypeptide comprising the amino acid sequence of SEQ ID NO:2 (also referred to herein as "CD39L2"); or a polynucleotide encoding a polypeptide comprising amino acid residues 72-93, 147-162, 191-211 OR 217-238 of SEQ ID NO:2.

In selected embodiments, such isolated polynucleotides of the invention represents a polynucleotide comprising the
20 nucleotide sequence of SEQ ID NO:1; or a polynucleotide comprising nucleotides 232-1599, 445-510, 670-717, 802-864 or 880-945 of the nucleotide sequence of SEQ ID NO:1.

The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes to the complement of the nucleotide sequence of
25 SEQ ID NO:1 under highly stringent hybridization conditions; a polynucleotide that hybridizes to the complement of the nucleotide sequence of SEQ ID NO:1 under moderately stringent hybridization conditions; a polynucleotide which is an allelic variant of any polynucleotide recited above; a polynucleotide which encodes a species homologue of any of
30 the proteins recited above; or a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptide of SEQ ID NO:2.

include use as hybridization probes, use as primers for PCR, use for chromosome and gene mapping, use in the recombinant production of protein, and use in generation of anti-sense DNA or RNA, their chemical analogs and the like. For
5 example, when the expression of an mRNA is largely restricted to a particular cell or tissue type, polynucleotides of the invention can be used as hybridization probes to detect the presence of the particular cell or tissue mRNA in a sample using, e.g., in situ hybridization.

In other exemplary embodiments, the polynucleotides are
10 used in diagnostics as expressed sequence tags for identifying expressed genes or, as well known in the art and exemplified by Vollrath et al., Science 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

The polypeptides according to the invention can be used
15 in a variety of conventional procedures and methods that are currently applied to other proteins. For example, the polypeptides of the invention can be used as molecular weight markers, and as a food supplement. In addition, a polypeptide of the invention can be used to generate an antibody that specifically binds the polypeptide.

20 Methods are also provided for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a protein of the present invention and a pharmaceutically acceptable carrier.

The polypeptides and polynucleotides of the invention
25 can be utilized, for example, as part of methods for modulating ecto-ATPase activity and for identifying compounds that can be utilized as part of methods for modulating ecto-ATPase activity. Among the processes that can be modulated via such methods are processes involved in cell adhesion, apoptosis, vesicular transport, signalling, including
30 purinergic, synaptic and neurotransmitter signalling, and purine recycling. The polypeptides of the invention having ADPase activity are also useful as anticoagulants and for inhibiting platelet aggregation. The polypeptides of the

invention can further be utilized as anti-thrombotic agents, anti-tissue graft rejection agents, and/or as part of methods for regulating neurotransmission by ATP in smooth muscle, peripheral ganglia or the brain.

5 The methods of the present invention further relate to methods for detecting the presence of the polynucleotides or polypeptides of the invention in a sample. Such methods can, for example, be utilized as part of prognostic and diagnostic evaluation of disorders as recited above and for the identification of subjects exhibiting a predisposition to
10 such conditions. Furthermore, the invention provides methods for evaluating the efficacy of drugs, and monitoring the progress of patients, involved in clinical trials for the treatment of disorders as recited above.

The invention also provides methods for the identification of compounds that modulate the expression of
15 the polynucleotides and/or polypeptides of the invention. Such methods can be utilized, for example, for the identification of compounds that can ameliorate symptoms of disorders as recited above. Such methods can include, but are not limited to, assays for identifying compounds and other substances that interact with (e.g., bind to) the
20 polypeptides of the invention.

The methods of the invention also include methods for the treatment of disorders as recited above which may involve the administration of such compounds to individuals exhibiting symptoms or tendencies related to disorders as recited above. In addition, the invention encompasses
25 methods for treating diseases or disorders as recited above by administering compounds and other substances that modulate the overall activity of the target gene products. Compounds and other substances can effect such modulation either on the level of target gene expression or target protein activity.

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4. BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Top line: Nucleotide sequence of SEQ ID NO:7, referred to herein as mNTPase or mCD39L4; bottom line:

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amino acid sequence of SEQ ID NO:8, referred to herein as mNTPase or mCD39L4.

Figure 2. Amino acid alignments of the full mNTPase (mCD39L4) amino acid sequence (SEQ ID NO:8) and the most closely related other NTPase proteins: garden pea NTPase (SEQ ID NO:10), potato apyrase (SEQ ID NO:11), yeast GDPase (SEQ ID NO:12). Identical residues are indicated by dark background (white letters), while conserved residues are indicated by gray background. Alignments were made with pileup and boxshade from the Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison WI.

Figure 3. Alignment of 12 members of the NTPase (or CD39-like) gene family indicating the conserved apyrase regions I-IV. CD39=human (from Accession No. S73813; SEQ ID NO:13), ratCD39=rat (from Accession No. gi11754710; SEQ ID NO:14), CD39L1=human (Accession No. U91510; SEQ ID NO:15), ChickATPase=chicken (from Accession No. U74467; SEQ ID NO:16), peaNTPase=garden pea (from Accession No. P52914; SEQ ID NO:10), potRROP1=potato RROP1 gene (from Accession No. gi11381633; SEQ ID NO:11), YGDA1+y71KD=yeast genes (from Accession Nos. sp1P32621 + sp1P40009; SEQ ID NO:12), hCD39L2=CD39L2, celegans=C. *Elegans* gene (from Accession No. gi11086594; SEQ ID NO:17). Identical residues are indicated by dark background (white letters), conserved residues are indicated by gray background. Alignments were made with pileup and boxshade from the Wisconsin Package 9.0, Genetics Computer Group (GCG), Madison, WI. Conserved portions of ACRs I-IV are boxed.

Figure 4. Top line: Nucleotide sequence of SEQ ID NO:1, referred to herein as CD39L2; bottom line: amino acid sequence of SEQ ID NO:2, referred to herein as CD39L2.

Figure 5. Comparison of the hydrophobicity predictions for the amino acid sequences of members of the human CD39-like gene family. Predictions were made using the Topred-II

1.1 program (Claros, M.G. & Von Hejine, G., 1994, Comput. Appl. Biosci. 10:685-686; putative setting=0.5; certain setting=1.0).

5 Figure 6. Top line: Nucleotide sequence of SEQ ID NO:3, referred to herein as CD39L3; bottom line: amino acid sequence of SEQ ID NO:4, referred to herein as CD39L3.

 Figure 7. Top line: Nucleotide sequence of SEQ ID NO:5, referred to herein as CD39L4; bottom line: amino acid
10 sequence of SEQ ID NO:6, referred to herein as CD39L4.

 Figure 8. Amino acid alignments of the full-length protein sequences for human members of the CD39-like gene family. CD39 (from Accession No. S73813; SEQ ID NO:13), CD39L1 (from Accession No. U91510; SEQ ID NO:15), CD39L2 (it
15 is noted that the CD39L2 polypeptide illustrated here depicts a derived amino acid sequence that is encoded from the ATG codon beginning at nucleotide 148 (see FIG. 4) and, therefore, includes an additional 28 amino acid residues N-terminal to those depicted in FIG. 4; this form of CD39L2 is also intended to be included within the scope of the present
20 invention), CD39L3, CD39L4.

 Identical residues are indicated by a black background (white letters), and conserved residues are indicated by a gray background. Spaces in the sequences are indicated by a dot. Apyrase regions (ACRs) are indicated by arrows, with conserved portions of ACRs I-IV are highlighted by the boxed
25 sections. Alignments were made using pileup and boxshade from the Wisconsin Package Version 9.0 Genetics Computer Group (GCG), Madison, WI.

 Figure 9. Amino acid sequence of dCD39L4 ("dNTPase"; SEQ ID NO:9) and alignment of the amino acid sequence with the
30 most closely related members of the CD39-like gene family. peaGDP, garden pea NTPase (from Accession No. P52194; SEQ ID NO:10); ptoapyrase, potato RROP1 gene (from Accession No. gill1381633; SEQ ID NO:11); CD39L2; CD39L4, and yGDPase, yeast

yGDA1 gene (from Accession No. sp1P32621; SEQ ID NO:12).
Apyrase regions (ACRs) are indicated by arrows, with
conserved portions of ACRs I-IV are highlighted by the boxed
sections.

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5. DETAILED DESCRIPTION OF THE INVENTION

5.1. Definitions

10 The term "nucleotide sequence" refers to a heteropolymer
of nucleotides or the sequence of these nucleotides. The
terms "nucleic acid" and "polynucleotide" are also used
interchangeably herein to refer to a heteropolymer of
nucleotides. Generally, nucleic acid segments provided by
this invention may be assembled from fragments of the genome
and short oligonucleotide linkers, or from a series of
oligonucleotides, or from individual nucleotides, to provide
15 a synthetic nucleic acid which is capable of being expressed
in a recombinant transcriptional unit comprising regulatory
elements derived from a microbial or viral operon, or a
eukaryotic gene. In alternate embodiments, a nucleotide
sequence, polynucleotide or nucleic acid can correspond to a
genomic sequence (e.g., can contain intron as well as exon
20 sequence) or cDNA sequences (that is, contains no intron
sequence).

The terms "oligonucleotide fragment" or a
"polynucleotide fragment", "portion," or "segment" is a
stretch of polypeptide nucleotide residues which is long
enough to use in polymerase chain reaction (PCR) or various
25 hybridization procedures to identify or amplify identical or
related parts of mRNA or DNA molecules.

The terms "oligonucleotides" or "nucleic acid probes"
are prepared based on the polynucleotide sequences provided
in the present invention. Oligonucleotides comprise portions
of such a polynucleotide sequence having at least about 15
30 nucleotides and usually at least about 20 nucleotides.
Nucleic acid probes comprise portions of such a
polynucleotide sequence having fewer nucleotides than about 6

kb, usually fewer than about 1 kb. After appropriate testing to eliminate false positives, these probes may, for example, be used to determine whether specific mRNA molecules are present in a cell or tissue or to isolate similar nucleic acid sequences from chromosomal DNA as described by Walsh et al. (Walsh, P.S. et al., 1992, PCR Methods Appl 1:241-250).

The term "probes" includes naturally occurring or recombinant or chemically synthesized single- or double-stranded nucleic acids. They may be labeled by nick translation, Klenow fill-in reaction, PCR or other methods well known in the art. Probes of the present invention, their preparation and/or labeling are elaborated in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY; or Ausubel, F.M. et al., 1989, Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, both of which are incorporated herein by reference in their entirety.

The "oligonucleotide fragment," "polynucleotide fragment," "portion," "segment," "oligonucleotide" or "nucleic acid probe" is at least about 15, and preferably at least about 50, 100, 200, 300, 400, 500, 600, 700 or 800 nucleotides in length.

20 The term "stringent" is used to refer to conditions that are commonly understood in the art as stringent. Stringent conditions can include highly stringent conditions (i.e., hybridization to filter-bound DNA under in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65° C, and washing in 0.1xSSC/0.1% SDS at 68° C), and moderately stringent
25 conditions (i.e., washing in 0.2xSSC/0.1% SDS at 42° C).

In instances wherein hybridization of deoxyoligonucleotides is concerned, additional exemplary highly stringent hybridization conditions include washing in 6xSSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos).

The term "recombinant," when used herein to refer to a polypeptide or protein, means that a polypeptide or protein

is derived from recombinant (e.g., microbial or mammalian) expression systems. "Microbial" refers to recombinant polypeptides or proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product, "recombinant microbial" defines a polypeptide or protein essentially free of native endogenous substances and unaccompanied by associated native glycosylation. Polypeptides or proteins expressed in most bacterial cultures, e.g., *E. coli*, will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern in general different from those expressed in mammalian cells.

The term "recombinant expression vehicle or vector" refers to a plasmid or phage or virus or vector, for expressing a polypeptide from a DNA (RNA) sequence. An expression vehicle can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

25 The term "recombinant expression system" means host cells which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit extrachromosomally. Recombinant expression systems as defined herein will express heterologous polypeptides or proteins upon induction of the

30 regulatory elements linked to the DNA segment or synthetic gene to be expressed. This term also means host cells which have stably integrated a recombinant genetic element or elements having a regulatory role in gene expression, for

the ability of the polypeptide to exhibit an immunological activity. A polypeptide exhibits an "immunological activity" when antibodies can be generated that are directed against the polypeptide.

5 The term "naturally occurring polypeptide" refers to polypeptides produced by cells that have not been genetically engineered and specifically contemplates various polypeptides arising from post-translational modifications of the polypeptide including, but not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and
10 acylation.

The term "mature" refers to a polypeptide that has been postranslationally modified or that corresponds in primary amino acid sequence to a polypeptide that has been postranslationally modified. A mature polypeptide includes, but is not limited to, a polypeptide which comprises a
15 primary amino acid sequence that has been processed from a "pre-," "pro-," or "pre-pro" amino acid sequence; a polypeptide which comprises a primary amino acid sequence corresponding to that of a polypeptide that has been processed from a "pre-," "pro-," or "pre-pro" amino acid
20 modified via such modifications as, for example, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

The term "derivative" refers to polypeptides chemically modified by such techniques as ubiquitination, labeling (e.g., with radionuclides or various enzymes), pegylation
25 (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of amino acids such as ornithine, which do not normally occur in human proteins.

The term "recombinant variant" refers to any polypeptide differing from naturally occurring polypeptides by amino acid insertions, deletions, and substitutions, created using
30 recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest, such as cellular trafficking, may be found by comparing the sequence of the

equivalent sequence can be routinely identified by applying the foregoing algorithm.

In yet another alternate embodiment, a substantially equivalent sequence of the invention varies from a listed sequences by no more than 2%, i.e., the number of individual residue substitutions, additions, and/or deletions in a substantially equivalent sequence, as compared to the corresponding reference sequence, divided by the total number of residues in the substantially equivalent sequence is about 0.02 or less. Such a sequence is said to have 98% sequence identity to the listed sequence. Such a substantially equivalent sequence can be routinely identified by applying the foregoing algorithm.

Substantially equivalent, e.g., mutant, amino acid sequences according to the invention generally have at least 95% sequence identity with a listed amino acid sequence, whereas substantially equivalent nucleotide sequence of the invention can have lower percent sequence identities, taking into account, for example, the redundancy or degeneracy of the genetic code. For the purposes of the present invention, sequences having substantially equivalent biological activity and substantially equivalent expression characteristics are considered substantially equivalent. In a preferred embodiment, for the purposes of determining equivalence, truncation of the mature sequence (e.g., via a mutation which creates a spurious stop codon) are disregarded.

Nucleic acid sequences encoding such substantially equivalent sequences, e.g., sequences of the recited percent identities, can also routinely be isolated and identified via standard hybridization procedures well known to those of skill in the art.

Where desired, an expression vector may be designed to contain a "signal or leader sequence" which will direct the polypeptide through the membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous protein sources by recombinant DNA techniques.

5 A polypeptide "fragment," "portion," or "segment" is a stretch of amino acid residues of at least about 5 amino acids, often at least about 7 amino acids or about at least about 9 to 13 amino acids, and, in various embodiments, at least about 17, 25, 50, 75, 100, 150, 200, 300, 400 or more amino acids. To be "active," any polypeptide must have sufficient length to display biologic and/or immunologic activity.

10 Recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the
15 polypeptide or domains of other peptides added to the polypeptide to modify the properties of any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate. Such variant nucleic acids and polypeptides are to be considered part of the present invention.

20 The term "activated" cells as used herein refers to those cells that are engaged in extracellular or intracellular membrane trafficking, including the export of neurosecretory or enzymatic molecules as part of a normal or disease process.

The term "purified" as used herein denotes that the
25 indicated nucleic acid or polypeptide is present in the substantial absence of other biological macromolecules, e.g., polynucleotides, proteins, and the like. In one embodiment, the polynucleotide or polypeptide is purified such that it constitutes at least 95% by weight, more preferably at least 99.8% by weight, of the indicated biological macromolecules
30 present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000 daltons, can be present).

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POLYNUCLEOTIDES AND NUCLEIC ACIDS OF THE INVENTION

Nucleotide and amino acid sequences of the invention are reported below. Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of
 5 the protein may be in linear form or they may be cyclized using known methods, for example, as described in H. U. Saragovi, et al., Bio/Technology 10, 773-778 (1992) and in R. S. McDowell, et al., J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as
 10 immunoglobulins for many purposes, including increasing the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to
 15 generate such fusions. For example, a protein--IgM fusion would generate a decavalent form of the protein of the invention.

The present invention also provides both full-length and mature forms of the disclosed proteins. The full-length forms of the polypeptides of the invention are identified in the
 20 figures and the sequence listing by translation of the nucleotide sequence of each nucleic acid molecule. Mature forms of the polypeptides of the invention can routinely be obtained by expression of the disclosed nucleotides encoding the full-length polypeptides in a suitable mammalian cell or other host cell. The sequence of the mature forms of
 25 the polypeptides can also routinely be determined from the amino acid sequence of the full-length polypeptides.

The present invention also provides genes corresponding to cDNA sequences disclosed herein. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include
 30 the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials.

Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. In such forms part or all of the intracellular and transmembrane domains of
5 the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information.

10 Species homologs of the disclosed polynucleotides and proteins are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species. Species homologs can include, but are not limited
15 to human, murine, rat or *Drosophila* species homologs.

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous or related to that encoded by the polynucleotides. Sequences
20 and allelic variant sequences of the invention can include, but are not limited to human, murine, rat and *Drosophila* sequences.

The compositions of the present invention include isolated polynucleotides, including recombinant DNA molecules, cloned genes or degenerate variants thereof,
25 especially naturally occurring variants such as allelic variants, novel isolated polypeptides, and antibodies that specifically recognize one or more epitopes present on such polypeptides.

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5.2. Nucleic Acids of the Invention

The isolated polynucleotides of the invention include, but are not limited to, a polynucleotide encoding a

The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes to the complement of the nucleotide sequence of SEQ ID NO:3 under highly stringent hybridization conditions; a polynucleotide that hybridizes to the complement of the nucleotide sequence of SEQ ID NO:3 under moderately stringent hybridization conditions; a polynucleotide which is an allelic variant of any polynucleotide recited above; a polynucleotide which encodes a species homologue of any of the proteins recited above; or a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptide of SEQ ID NO:4. Such polynucleotides hybridize under the above conditions to the complement of SEQ ID NO:3 or to a fragment of SEQ ID NO:3, wherein the fragment is greater than at least about 10 bp, and, in alternate embodiments, is about 20 to about 50 bp, or is greater than about 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, or 800 bp.

The isolated polynucleotides of the invention still further include, but are not limited to, a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:6 (also referred to herein as "CD39L4"); or a
20 polynucleotide encoding a polypeptide comprising amino acid residues 47-68, 123-138, 167-187 or 193-214 of SEQ ID NO:6; or a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:9 (also referred to herein as dCD39L4"); or a polynucleotide encoding amino acid residues 77-98, 153-167, 197-217 or 223-242 of SEQ ID NO:9.

The polynucleotides of the present invention also
30 include, but are not limited to, a polynucleotide that
hybridizes to the complement of the nucleotide sequence of
SEQ ID NO:5 under highly stringent hybridization conditions;
a polynucleotide that hybridizes to the complement of the

nucleotide sequence of SEQ ID NO:5 under moderately stringent hybridization conditions; a polynucleotide which is an allelic variant of any polynucleotide recited above; a polynucleotide which encodes a species homologue of any of the proteins recited above; or a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptide of SEQ ID NO:6. Such polynucleotides hybridize under the above conditions to the complement of SEQ ID NO:5 or to a fragment of SEQ ID NO:5, wherein the fragment is greater than at least about 10 bp, and, in alternate embodiments, is about 20 to about 50 bp, or is greater than about 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp or 800 bp.

The isolated polynucleotides of the invention further include, but are not limited to, a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:8 (also referred to herein as "mCD39L4" or "mNTPase"); or a polynucleotide encoding amino acid residues 46-67, 122-140, 166-187 or 194-213 of SEQ ID NO:8.

In selected embodiments, such isolated polynucleotides of the invention represents a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7; or a polynucleotide comprising nucleotides 205-1599, 340-395, 568-624, 700-765 or 784-843 of the nucleotide sequence of SEQ ID NO:7.

The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes to the complement of the nucleotide sequence of SEQ ID NO:7 under highly stringent hybridization conditions; 25 a polynucleotide that hybridizes to the complement of the nucleotide sequence of SEQ ID NO:7 under moderately stringent hybridization conditions; a polynucleotide which is an allelic variant of any polynucleotide recited above; a polynucleotide which encodes a species homologue of any of the proteins recited above; or a polynucleotide that encodes 30 a polypeptide comprising a specific domain or truncation of the polypeptide of SEQ ID NO:8. Such polynucleotides hybridize under the above conditions to the complement of SEQ ID NO:7 or to a fragment of SEQ ID NO:7, wherein the fragment

about 0.05 or less. Such a sequence is said to have 95% sequence identity to the listed sequence. Such a substantially equivalent sequence can be routinely identified by applying the foregoing algorithm.

5 In yet another alternate embodiment, a substantially equivalent sequence of the invention varies from a listed sequences by no more than 2%, i.e., the number of individual nucleotide substitutions, additions, and/or deletions in a substantially equivalent sequence, as compared to the corresponding reference sequence, divided by the total number
10 of nucleotides in the substantially equivalent sequence is about 0.02 or less. Such a sequence is said to have 98% sequence identity to the listed sequence. Such a substantially equivalent sequence can be routinely identified.

A polynucleotide according to the invention can be
15 joined to any of a variety of other nucleotide sequences by well-established recombinant DNA techniques (see Sambrook J et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY). Useful nucleotide sequences for joining to polypeptides include an assortment of vectors, e.g., plasmids, cosmids, lambda phage derivatives, phagemids,
20 and the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable
25 marker for the host cell. Vectors according to the invention include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. A host cell according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

30 The sequences falling within the scope of the present invention are not limited to the specific sequences herein described, but also include allelic variations thereof. Allelic variations can be routinely determined by comparing

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(e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

The nucleic acid sequences of the invention are further directed to sequences which encode variants of the described nucleic acids. These amino acid sequence variants may be prepared by methods known in the art by introducing appropriate nucleotide changes into a native or variant polynucleotide. There are two variables in the construction of amino acid sequence variants: the location of the mutation and the nature of the mutation. The amino acid sequence variants of the nucleic acids are preferably constructed by mutating the polynucleotide to give an amino acid sequence that does not occur in nature. These amino acid alterations can be made at sites that differ in the nucleic acids from different species (variable positions) or in highly conserved regions (constant regions). Sites at such locations will typically be modified in series, e.g., by substituting first with conservative choices (e.g., hydrophobic amino acid to a different hydrophobic amino acid) and then with more distant choices (e.g., hydrophobic amino acid to a charged amino acid), and then deletions or insertions may be made at the target site. Amino acid sequence deletions generally range from about 1 to 30 residues, preferably about 1 to 10 residues, and are typically contiguous. Amino acid insertions include amino- and/or carboxyl-terminal fusions ranging in length from one to one hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions may range generally from about 1 to 10 amino residues, preferably from 1 to 5 residues. Examples of terminal insertions include the heterologous signal sequences necessary for secretion or for intracellular targeting in different host cells.

Finally, it is to be understood that the nucleic acid molecules of the invention further include any nucleic acid molecule that encodes the polypeptides of the invention, as described in Section 5.4, below.

5

5.3. Hosts

The present invention further provides host cells genetically engineered to contain the polynucleotides of the invention. For example, such host cells may contain nucleic acids of the invention introduced into the host cell using
10 known transformation, transfection or infection methods. The present invention still further provides host cells genetically engineered to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in
15 the cell.

The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium
20 phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L. et al., *Basic Methods in Molecular Biology* (1986)). The host cells containing one of polynucleotides of the invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF) or can be used to produce a
25 heterologous protein under the control of the EMF.

Any host/vector system can be used to express one or more of the ORFs of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, Cv-1 cell, COS cells, and Sf9 cells, as well as prokaryotic host such as *E. coli* and *B. subtilis*. The most preferred
30 cells are those which do not normally express the particular polypeptide or protein or which expresses the polypeptide or protein at low natural level. Mature proteins can be

International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

5

5.4. Polypeptides of the Invention

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising the amino acid sequence of SEQ ID NO:2; or a polypeptide comprising amino acid residues 72-93, 147-162, 191-211 OR 217-238 of SEQ ID NO:2.

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising the amino acid sequence of SEQ ID NO:4; or a polypeptide comprising amino acid residues 55-76, 132-150, 179-199 or 213-234 of SEQ ID NO:4.

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising the amino acid sequence of SEQ ID NO:6; or a polypeptide comprising amino acid residues 47-68, 123-138, 167-187 or 193-214 of SEQ ID NO:6.

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising the amino acid sequence of SEQ ID NO:8; or a polypeptide comprising amino acid residues 46-67, 122-140, 166-187 or 194-213 of SEQ ID NO:8.

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising the amino acid sequence of SEQ ID NO:9; or a polypeptide comprising amino acid residues 77-98, 153-167, 197-217 or 223-242 of SEQ ID NO:9.

The isolated polypeptides of the invention further include polypeptides that are substantially equivalent to the polypeptides of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:9 or to specific domains thereof.

Typically, such a substantially equivalent sequence varies from one of those listed herein by no more than about 20%, i.e., the number of individual amino acid residue

have 98% sequence identity to the listed sequence. Such a substantially equivalent sequence can be routinely identified.

Preferred embodiments include those in which the protein
5 produced by such process is a mature form of the protein.

Protein compositions of the present invention may further comprise an acceptable carrier, such as a hydrophilic, e.g., pharmaceutically acceptable, carrier.

The invention also relates to methods for producing a polypeptide comprising growing a culture of the cells of the
10 invention in a suitable culture medium, and purifying the protein from the culture. For example, the methods of the invention include a process for producing a polypeptide in which a host cell containing a suitable expression vector that includes a polynucleotide of the invention is cultured under conditions that allow expression of the encoded
15 polypeptide. The polypeptide can be recovered from the culture, conveniently from the culture medium, and further purified.

The present invention further provides isolated polypeptides encoded by the nucleic acid fragments of the present invention or by degenerate variants of the nucleic
20 acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (e.g., an ORF) by nucleotide sequence but, due to the degeneracy of the genetic code, encode an identical polypeptide sequence. Preferred nucleic acid fragments of the present invention are
25 the ORFs that encode proteins.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. This is particularly useful
30 in producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating antibodies against the native polypeptide. In an alternative method, the polypeptide or protein is purified

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from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily follow known methods for isolating polypeptides and proteins in order to obtain one of the isolated polypeptides or proteins of the present invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immuno-affinity chromatography. See, e.g., Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag (1994); Sambrook, et al., in *Molecular Cloning: A Laboratory Manual*; Ausubel et al., *Current Protocols in Molecular Biology*.

The polypeptides and proteins of the present invention can alternatively be purified from cells which have been altered to express the desired polypeptide or protein. As used herein, a cell is said to be altered to express a desired polypeptide or protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not produce or which the cell normally produces at a lower level. One skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention. The purified polypeptides can be used in *in vitro* binding assays which are well known in the art to identify molecules which bind to the polypeptides. These molecules include but are not limited to, for e.g., small molecules, molecules from combinatorial libraries, antibodies or other proteins. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

In addition, the binding molecules may be complexed with toxins, e.g., ricin or cholera, or with other compounds that are toxic to cells. The toxin-binding molecule complex is

then targeted to the tumor or other cell by the specificity of the binding molecule for SEQ ID NO:2, SEQ ID NO:4 SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:9, or another of the polypeptide of the invention.

- 5 The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

- 10 The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common
15 therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

- 20 The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may
25 include the alteration, substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion
30 are well known to those skilled in the art (see, e.g., U.S. Pat. No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, Calif., U.S.A. (the MaxBat.RTM. kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl.RTM. or Cibacrom blue 3GA Sepharose.RTM.; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be
30 expressed in a form which will facilitate purification. For
example, it may be expressed as a fusion protein, such as
those of maltose binding protein (MBP), glutathione-S-
transferase (GST) or thioredoxin (TRX). Kits for expression

and purification of such fusion proteins are commercially available from New England BioLab (Beverly, Mass.), Pharmacia (Piscataway, N.J.) and In Vitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified
5 by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, Conn.).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or
10 other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the
15 present invention as an "isolated protein."

It is to be understood that polypeptides consisting of the following amino acid sequence are not considered part of the present invention: the amino acid sequence of, or, where appropriate, reported to be encoded by the nucleotide sequence of Genbank™ accession No :S73813, gi11754710,
20 U91510, U91511, AA116990, AA120757, HO8436, AA378537, AA336644, AA338117, AA337885, N72742, AA256016, AA611283, AA647051, AA638277, AA271520, W46136, AA391695, AA390461, AA201196, AA246996 or AA567512.

5.5. Uses And Biological Activity

25 The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration
30 or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

5.5.1. Research Uses and Utilities

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant

5 protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to

10 identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process

15 of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide

20 encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding

25 occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in

30 assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a

5.5.3. Cytokine and Cell Proliferation/
Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor-dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+(preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., I. Immunol. 149:3778-3783, 1992; Bowman et al., I. Immunol. 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A. M. and Shevach, E. M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human interleukin .gamma., Schreiber, R. D. In Current Protocols in Immunology. J. E.

e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

- Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without
- 5 limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L. S. and Lipsky, P. E. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988;
- 10 Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6--Nordan, R. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11--
- 15 Bennett, F., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9--Ciarletta, A., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.13.1, John
- 20 Wiley and Sons, Toronto. 1991.

- Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in
- 25 Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA
- 30 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

5.5.4. Immune Stimulating or Suppressing Activity

5 A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A polynucleotide of the invention can encode a polypeptide exhibiting such activities. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting
10 the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by vital (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein of
15 the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the
20 treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune
25 thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune
30 suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

- Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing
- 5 the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent.
 - 10 Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence
 - 15 of the tolerizing agent.

- Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ
- 20 transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The
 - 25 administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking
 - 30 antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter

blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-vital immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary

costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient mounts of

5 MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I α .alpha. chain protein and .beta..sub.2 microglobulin protein or an MHC class II .alpha. chain protein and an MHC class II .beta. chain protein to thereby express MHC class I or MHC

10 class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an

15 MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to

20 overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M.

- 25 Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J.
- 30 Immunol. 135:1564-1572, 1985; Takai et al., I. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982;

- Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowman et al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991;
- 5 Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol.

- 10 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J. J. and Brunswick, M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans);

- 20 Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those

- 25 described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994;
- 30 Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in:

5 Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

10 Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

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5.5.5. Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming

20 cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the

25 production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-

30 megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting

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the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually
5 treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or
10 heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

15 Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood
20 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lymphohematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M. G. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds.
25 Vol pp. 265-268, Wiley-Liss, Inc., New York, N.Y. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I. K. and Briddell, R. A. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, N.Y. 1994;
30 Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R. E. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, N.Y. 1994; Long

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5.5.6. Tissue Growth Activity

10 A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

15 A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an
20 osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract
25 bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase
30 activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention

is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or
 5 ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing
 10 defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons
 15 or ligaments. The compositions of the present invention may provide environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors ex vivo for return in vivo to effect tissue
 20 repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful
 25 for proliferation of neural cells and for regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the
 30 treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's

neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, H. I. and Rovee, D. T., eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

5.5.7. Activin/Inhibin Activity

A protein of the present invention may also exhibit activin- or inhibin-related activities. A polynucleotide of the invention may encode a polypeptide exhibiting such characteristics. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin α -family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin- β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, U.S. Pat. No. 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature

318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA
83:3091-3095, 1986.

5.5.8. Chemotactic/Chemokinetic Activity

5 A protein of the present invention may have chemotactic
or chemokinetic activity (e.g., act as a chemokine) for
mammalian cells, including, for example, monocytes,
fibroblasts, neutrophils, T-cells, mast cells, eosinophils,
epithelial and/or endothelial cells. A polynucleotide of the
invention can encode a polypeptide exhibiting such
10 attributes. Chemotactic and chemokinetic proteins can be
used to mobilize or attract a desired cell population to a
desired site of action. Chemotactic or chemokinetic proteins
provide particular advantages in treatment of wounds and
other trauma to tissues, as well as in treatment of localized
infections. For example, attraction of lymphocytes, monocytes
15 or neutrophils to tumors or sites of infection may result in
improved immune responses against the tumor or infecting
agent.

A protein or peptide has chemotactic activity for a
particular cell population if it can stimulate, directly or
indirectly, the directed orientation or movement of such cell
20 population. Preferably, the protein or peptide has the
ability to directly stimulate directed movement of cells.
Whether a particular protein has chemotactic activity for a
population of cells can be readily determined by employing
such protein or peptide in any known assay for cell
chemotaxis.

25 The activity of a protein of the invention may, among
other means, be measured by the following methods:

Assays for chemotactic activity (which will identify
proteins that induce or prevent chemotaxis) consist of assays
that measure the ability of a protein to induce the migration
of cells across a membrane as well as the ability of a
30 protein to induce the adhesion of one cell population to
another cell population. Suitable assays for movement and
adhesion include, without limitation, those described in:
Current Protocols in Immunology, Ed by J. E. Coligan, A. M.

Kruisbeek, D. H. Marguiles, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; 5 Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25:1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153:1762-1768, 1994.

5.5.9. Hemostatic and Thrombolytic Activity

10 A protein of the invention may also exhibit hemostatic or thrombolytic activity. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other

15 hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

20 The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

5.5.10. Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. A polynucleotide of the invention can encode a polypeptide exhibiting such characteristics. Examples of such receptors and ligands include, without limitation, cytokine receptors and their

ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, 5 integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the 10 present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include 15 without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; 20 Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

5.5.11. Anti-Inflammatory Activity

25 Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the 30 inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities

can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation intimation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)),
5 ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to
10 an antigenic substance or material.

5.5.12. Leukemias

Leukemias and related disorders may be treated or prevented by administration of a therapeutic that promotes or inhibits function of the polynucleotides and/or polypeptides
15 of the invention. Such leukemias and related disorders include but are not limited to acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia, chronic leukemia, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia (for
20 a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia).

5.5.13. Nervous System Disorders

Nervous system disorders, involving cell types which can be tested for efficacy of intervention with compounds that
25 modulate the activity of the polynucleotides and/or polypeptides of the invention, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination.
30 Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following

lesions of either the central (including spinal cord, brain) or peripheral nervous systems:

- 5 (i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;
- 10 (ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;
- 15 (iii) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;
- 20 (iv) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;
- 25 (v) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and
- 30 (vi) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic

lupus erythematosus, carcinoma, or sarcoidosis;

(vii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and

5 (viii) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

10
Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not
15 by way of limitation, therapeutics which elicit any of the following effects may be useful according to the invention:

(i) increased survival time of neurons in culture;
(ii) increased sprouting of neurons in culture or in vivo;

20 (iii) increased production of a neuron-associated molecule in culture or in vivo, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or

(iv) decreased symptoms of neuron dysfunction in vivo.

25 Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann.
30 Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., depending on the molecule to be measured; and motor neuron dysfunction

may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In a specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

5.5.14. Other Activities

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, co-factors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders),

depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; 5 hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen 10 in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

5.6. Pharmaceutical Formulations and Routes of Administration

15 A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be administered to a patient in need, by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at doses to treat or ameliorate a variety of disorders. Such a 20 composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The 25 characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg- 30 CSF, thrombopoietin, stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or compliment its activity or use in treatment. Such additional

5 factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Conversely, protein of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent. A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

15 Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms, e.g., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

30 In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic

5 factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

10 5.6.1. Routes of Administration

Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a arthritic joints or in fibrotic tissue, often in a depot or sustained release formulation.

25 In order to prevent the scarring process frequently occurring as complication of glaucoma surgery, the compounds may be administered topically, for example, as eye drops. Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a specific antibody, targeting, for example, arthritic or fibrotic tissue. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

5.6.2. Compositions/Formulations

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. These pharmaceutical compositions may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective amount of protein of the present invention is administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably from about 25 to 90% protein of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.

When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally

acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art.

REF ID: A65300

a salt thereof such as sodium alginate. Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

- Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

- For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection

alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The cosolvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g. polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose. Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars,

of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Pat. Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

5 The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention
10 with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not
15 increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 μ g to about 100 mg (preferably about 0.1 μ g to about 10 mg, more preferably about 0.1 μ g to about 1 mg) of protein of the present invention per kg body weight. For compositions of the
20 present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically
25 acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention which may also optionally be included in the
30 composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the

composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed
5 into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate
10 formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are
15 comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or
20 collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability. Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging
25 from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including
30 hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic

and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced
5 either in vivo or ex vivo into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA). Cells may also be cultured ex vivo in the presence of
10 proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced in vivo for therapeutic purposes.

5.6.3. Effective Dosage

15 Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the
20 subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be
25 formulated in animal models to achieve a circulating concentration range that includes the IC₅₀ as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal inhibition of the C-proteinase activity). Such information can be used to more accurately determine useful doses in humans.

30 A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and

therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD₅₀ and ED₅₀. Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. See, e.g., Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1. Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the C-proteinase inhibiting effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from in vitro data; for example, the concentration necessary to achieve 50-90% inhibition of the C-proteinase using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

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The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

5.6.4. Packaging

10 The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labelled for treatment of an indicated condition.

15

5.7. Antibodies

20 Another aspect of the invention is an antibody that specifically binds the polypeptide of the invention. Such antibodies can be either monoclonal or polyclonal antibodies, as well fragments thereof and humanized forms or fully human forms, such as those produced in transgenic animals. The invention further provides a hybridoma that produces an antibody according to the invention. Antibodies of the invention are useful for detection and/or purification of the polypeptides of the invention.

25 Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in
30 the art, for example, as in R. P. Merrifield, J. Amer. Chem. Soc. 85, 2149-2154 (1963); J. L. Krstenansky, et al., FEBS

heterologous protein (such as globulin or β -galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells. Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz et al., *Exp. Cell Research*. 175:109-124 (1988)).

Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, A.M., *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1984)). Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to proteins of the present invention.

For polyclonal antibodies, antibody containing antiserum is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures. The present invention further provides the above-described antibodies in delectably labeled form. Antibodies can be delectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, etc.) fluorescent labels (such as FITC or rhodamine, etc.), paramagnetic atoms, etc. Procedures for accomplishing such labeling are well-known in the art, for example, see (Sternberger, L.A. et al., *J. Histochem. Cytochem.* 18:315 (1970); Bayer, E.A. et al., *Meth. Enzym.* 62:308 (1979); Engval, E. et al., *Immunol.* 109:129 (1972); Goding, J.W. *J. Immunol. Meth.* 13:215 (1976)).

artisan can readily adopt any of the presently known methods for recording information on computer readable medium to generate manufactures comprising the nucleotide sequence information of the present invention.

- 5 A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor
- 10 programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a
- 15 database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of dataprocessor structuring formats (e.g. text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention. By providing the nucleotide sequence of
- 20 SEQ ID NO:1 or a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NO:1 in computer readable form, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a
- 25 computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul et al., *J. Mol. Biol.* 215:403-410 (1990)) and BLAZE (Brutlag et al., *Comp. Chem.* 17:203-207 (1993)) search algorithms on a Sybase system is used to identify open reading frames (ORFs) within a nucleic acid sequence. Such ORFs may be protein
- 30 encoding fragments and may be useful in producing commercially important proteins such as enzymes used in

fermentation reactions and in the production of commercially useful metabolites.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based systems are suitable for use in the present invention. As stated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory which can store nucleotide sequence information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention.

As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of a known sequence which match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software includes, but is not limited to, MacPattern (EMBL), BLASTN and BLASTA (NPOLYPEPTIDEIA). A skilled artisan can readily recognize that any one of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present computer-based systems. As used herein, a "target sequence" can be any nucleic acid or amino acid sequence of six or more nucleotides or two or more amino acids. A

transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide.

5.10. Diagnostic Assays and Kits

The present invention further provides methods to identify the presence or expression of one of the ORFs of the present invention, or homolog thereof, in a test sample, using a nucleic acid probe or antibodies of the present invention.

In general, methods for detecting a polynucleotide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polynucleotide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polynucleotide of the invention is detected in the sample.

Such methods can also comprise contacting a sample under stringent hybridization conditions with nucleic acid primers that anneal to a polynucleotide of the invention under such conditions, and amplifying annealed polynucleotides, so that if a polynucleotide is amplified, a polynucleotide of the invention is detected in the sample.

In general, methods for detecting a polypeptide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polypeptide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polypeptide of the invention is detected in the sample.

In detail, such methods comprise incubating a test sample with one or more of the antibodies or one or more of nucleic acid probes of the present invention and assaying for binding of the nucleic acid probes or antibodies to components within the test sample.

Conditions for incubating a nucleic acid probe or antibody with a test sample vary. Incubation conditions

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Academic Press, San Diego. Polypeptides may be expressed in a variety of host cells, either prokaryotic or eukaryotic. Host cells may be from the same species from which a particular polypeptide nucleotide sequence was isolated or
5 from a different species. Advantages of producing polypeptides by recombinant DNA technology include obtaining adequate amounts of the protein for purification and the availability of simplified purification procedures.

Each sequence so obtained was compared to sequences in GenBank using a search algorithm developed by Applied
10 Biosystems and incorporated into the INHERIT[™] 670 Sequence Analysis System. In this algorithm, Pattern Specification Language (developed by TRW Inc., Los Angeles, CA) was used to determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of
15 these three parameters, the DNA database was searched for sequences containing regions of homology to the query sequence, and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman
20 alignments were used to display the results of the homology search. Peptide and protein sequence homologies were ascertained using the INHERIT[™] 670 Sequence Analysis System in a way similar to that used in DNA sequence homologies. Pattern Specification Language and parameter windows were used to search protein databases for sequences containing
25 regions of homology that were scored with an initial value. Dot-matrix homology plots were examined to distinguish regions of significant homology from chance matches.

Alternatively, BLAST, which stands for Basic Local Alignment Search Tool, is used to search for local sequence alignments (Altschul SF (1993) J Mol Evol 36:290-300;
30 Altschul, SF et al (1990) J Mol Biol 215:403-10). BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in

Hydropathy plots with Topred-II 1.1 (Claros, M.G. & Von Hejine, G., 1994, Comput. Appl. Biosci. 10:685-686) predict a single potential transmembrane segment close to the amino terminus of the protein, suggesting a single-pass transmembrane protein with a large extracellular domain. Two potential glycosylation sites can be found at amino acid positions 41 (NVSA) and at 231 (NSTF), suggesting that mNTPase is glycosylated.

Database searches with the derived amino acid sequence identified homology with other members of the NTPase family. FIG. 2 shows an alignment of the full mNTPase (mCD39L4) protein sequence against three of the most homologous known NTPases, from garden pea, potato and *Saccharomyces cerevisiae*. The mNTPase protein shares approximately 30% amino acid identity with the three other NTPases.

The region of highest homology between all members of the NTPase family is at the amino terminus of the protein. Handa & Guidotti (Handa, M. & Guidotti, G., 1996, Biochem. Biophys. Res. Commun. 218:916-923) highlighted four regions of NTPases referred to as putative apyrase-conserved regions ("ACRs"). FIG. 3 shows an alignment of ACRs I-IV. (See Section 3, Section 5, and its subsections, above, for a delineation of the amino acid residues that make up ACRs I-IV of the CD39-like polypeptides of the invention.) ACR conservation would indicate that these regions are essential for the functioning of the protein, while changes in the regions surrounding these domains can be tolerated. The presence of all four ACRs in the mNTPase (mCD39L2) indicates that mNTPase is a new member of the NTPase family.

BLAST searches with the DNA sequence of mNTPase (mCD39L4) revealed two overlapping human EST clones with 57% DNA sequence identity to portions of mNTPase (Accession Nos. H08436 and AA378537). Upon combination and analysis of the resulting sequence, an ORF was identified that showed

30 homology to NTPases. The putative NTPase protein sequence, referred to herein as "CD39L2," is shown in FIG. 3 alongside the other NTPase protein sequences. The identification and

characterization of the full-length CD39L2 polypeptide and nucleotide sequences is described in the Example presented in Section 7, below.

To map the murine mNTPase gene, a cosmid was isolated from a mouse cosmid library, and used for fluorescence in situ hybridization (FISH). For the FISH analysis, slides with mouse metaphase chromosomes were prepared from spleens as described in Monier et al. (Monier, K. et al., 1996, Cytogenet. Cell Genet. 72:200-204). 1 microgram of mouse cosmid containing the mNTPase gene was labeled with biotin 14-ATP and a Bionick Kit (GibcoBRL). DNA was purified by passage through a Sephadex G50 column and ethanol precipitated with 50 micrograms of sheared salmon sperm DNA and tRNA. 80 ng of probe was dried down with 3 micrograms of mouse Cot-1 DNA (GibcoBRL). Hybridization was carried out as described (Ragoussis, J. et al., 1992, Genomics 14:423-430). Confirmation of chromosomal location was achieved by rehybridizing the same slide with a mouse Chromosome 12-specific Starfish Paint (Cambio).

The FISH study revealed the presence of mNTPase on mouse Chr. 12 at chromosome band E. To confirm the location of the mNTPase gene on mouse Chr. 12, linkage analysis was carried out upon the European Collaborative Interspecific Backcross (EUCIB). PCR primers were designed to the 3' untranslated region of the mNTPase cDNA sequence and used for PCR by use of mouse genomic DNA from the two parental mouse strains, *Mus spretus* and C57BL/6. A polymorphism was detected between the two strains by SSCP analysis and was used for the mapping. (PCR conditions: 48°C 20 sec., Primer 1: CCAGACTGTAAATCTTTTGG; Primer 2: AGGGAATGTAATAAGGGTAG; conditions: 94°C 2 minutes; 35 cycles of 94°C 20 sec. 72°C 20 sec., 72°C 1 min; product size: 320 bp).

Linkage with a LOD score of 8.14 was obtained with the genetic marker D12Mit4, flanked by D12Mit149 and D12Mit238, between 31.7 cM and 36.1 cM from the top of the mouse Chr. 12 linkage group for the MIT F₂ Intercross. This region of mouse Chr. 12 has previously been shown to share synteny with human

Chr. 14q (DeBry, R.W. & Seldin, M.F., 1996, Genomics 33:337-351).

5 7. **EXAMPLE: Identification and Characterization of
Additional Novel CD39-Like Polypeptides
and Nucleic Acid Molecules**

Described herein is the cloning and
characterization of novel CD-39-like nucleotide-
triphosphatase ("NTPase") gene and polypeptide sequences.

10 These sequences are referred to below as "CD39L2," "CD39L3,"
"CD39L4" and "dCD39L4."

7.1. Materials and Methods

Identification, isolation, and sequencing of cDNA clones
for CD39L2, CD39L3, and CD39L4. The nucleotide sequence of
CD39 (Accession No. S73813), CD39L1 (Accession No. U91510),
and mNTPase (see Section 6, above) were used in TBLASTX
searches against entries in the expressed sequence tag (EST)
database at EMBL/GenBank, using the Bork server
(<http://www.bork.embl-heidelberg.de/>). cDNA clones for
homologous IMAGE EST entries were obtained from the Human
20 Genome Mapping Project Resource Centre (HGMP, Hinxton, UK).
DNA was prepared with QiaTip-100 (Qiagen), and the cDNA was
sequenced by primer walking with a fluorescence labeled dye-
terminator cycle sequencing kit according to the
manufacturer's instructions (PRISM Ready Dye-Deoxy Terminator
Premix from Applied Biosystems Inc.) and electrophoresed on
25 an ABI 373 (Perkin-Elmer). Overlapping EST clones were
identified by searching with the nucleotide sequence against
entries in the EST database using BLAST-N
([http://www.ncbi.nlm.nih.gov:80/cgi-bin/BLAST/nph-
blast?Jform](http://www.ncbi.nlm.nih.gov:80/cgi-bin/BLAST/nph-blast?Jform)).

Additional IMAGE cDNA clones were ordered from HGMP if
30 they extended the existing nucleotide sequence further 5'.
cDNA clones corresponding to the most 5' extreme of each gene
were identified by hybridization of radiolabeled inserts of
IMAGE cDNA clones to a keratinocyte stem cell cDNA library, a

Figure 1. The 12 test items of the TAP. The items are arranged in a 4x3 grid. Each item consists of a diagram of a mechanical system (a spring-mass-damper system) and a corresponding differential equation. The items are labeled 1 through 12. The diagrams show a mass m connected to a wall by a spring with constant k and a damper with coefficient c . The displacement x is measured from the equilibrium position. The equations are:

- $m\ddot{x} + c\dot{x} + kx = 0$
- $m\ddot{x} + c\dot{x} + kx = F_0 \cos(\omega t)$
- $m\ddot{x} + c\dot{x} + kx = F_0 \sin(\omega t)$
- $m\ddot{x} + c\dot{x} + kx = F_0 \cos(\omega t) + F_0 \sin(\omega t)$
- $m\ddot{x} + c\dot{x} + kx = F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t)$
- $m\ddot{x} + c\dot{x} + kx = F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t)$
- $m\ddot{x} + c\dot{x} + kx = F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t)$
- $m\ddot{x} + c\dot{x} + kx = F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t)$
- $m\ddot{x} + c\dot{x} + kx = F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t)$
- $m\ddot{x} + c\dot{x} + kx = F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t)$
- $m\ddot{x} + c\dot{x} + kx = F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t)$
- $m\ddot{x} + c\dot{x} + kx = F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t) + F_0 \cos(\omega t) + F_0 \sin(\omega t)$

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Mapping of CD39L2, CD39L3, and CD39L4. Members of the CD39-like gene family were mapped in the human genome by PCR screening of the GeneBridge-4 radiation hybrid mapping panel obtained from the HGMP Resource Centre (Hinxton, UK) (Gyapay, G. et al., 1996, Hum. Mol. Genet. 5:339-346). PCR-positive radiation hybrid clones were organized into the GeneBridge-4 HGMP-RC subset order using the HGMP radiation hybrid mapping World Wide Web (WWW) site (<http://www.hgmp.mrc.ac.uk/cgi-bin/contig/contig/rhmapper.pl>), and mapping data for each gene were obtained from the Whitehead server (<http://www.genome.wi.mit.edu/cgi-bin/rhmapper.pl>). The chromosomal location for each gene was confirmed by PCR screening of the monochromosomal hybrids obtained from the HGMP Resource Centre. PCR primers were designed for the 3' untranslated region (UTR) of each gene and titrated for a unique human-specific PCR product. PCR conditions: CD39L2, Primer 1, 5'-CTGCTTGAGTGACCTCTCTG-3'; Primer 2, 5'-CACATGAGGTTTCAGCTCGTG-3'; 94°C for 2 min; 38 cycles of 94°C for 20 s, 54°C for 20 s, 72°C for 20 s; 72°C for 2 min. Product size is 362 bp). CD39L3, Primer 1: 5'-GTGAAGTGGCTGCCTTCAGG-3'; Primer 2, 5'-CCTTTGACTCGGGACTCCAG-3'; 94°C for 2 min; 38 cycles of 94°C for 20 s, 56°C for 20 s, 72°C for 2 min. Product size is 281 bp). CD39L4. Primer 1, 5'-GAAGTCTGCCTAACCACTC-3'; Primer 2, 5'-

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ATTGATGGGTCTTGGGATTGC-3'; 94°C 2 for min; 38 cycles of 94°C for 20 s, 56°C for 20 s; 72°C for 20 s; 72°C for 2 min. Product size is 234 bp. PCR products were analyzed by electrophoresis through 3.5% NuSieve agarose gels (Flowgen).

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7.2. Results

Isolation and Sequence Characterization of CD39L2.

Identification of partial human CD39L2 sequence was described in the Example presented in Section 6, above. The CD39L2 insert was used to isolate additional clones from a human adult breast epithelial cDNA library (ZR75), a human T-leukemia cell line J6 cDNA library (Jurkat), and a human keratinocyte stem cell cDNA library (KER). Of 23 cDNA clones that were isolated and sequenced, all but one appeared to be alternatively spliced or unspliced. Within the 2762 bp cDNA that appeared to be neither unspliced or alternatively spliced, an ORF extending to nucleotide 1600 containing ACRs I-IV was identified. Two ATG codons with a poor match to the consensus translation initiation site were found at nucleotide positions 148 and 232 (AUGUGAAUGA at 148 and ACAAGGAUGA at 232 versus consensus GCCGCCAUGG; Kozak, M., 1989, J. Biol. Chem. 108:229-241). Based on homology to mNTPase, the ATG at nucleotide position 232 is the initiation codon. (See FIG. 9 for a depiction of the CD39L2 amino acid sequence that results from translation from the upstream, position 148, start codon; such a form of CD39L2 as well as nucleotide sequences that encode this form of the polypeptide are also intended to be included as part of the present invention.) A single polyadenylation signal of AAUAAA was identified at nucleotide position 2700, 22 nucleotides 5' of the poly(A) tail of the human CD39L2 cDNA.

The nucleotide sequence (SEQ ID NO:1) and derived amino acid sequence (SEQ ID NO:2) of human CD39L2 is depicted in FIG. 4. Hydrophobicity plots using Topred-II 1.1 (Claros, M.G. & Von Hejine, G., 1994, Comput. Appl. Biosci. 10:685-686) predicted a single transmembrane segment at the N-terminal extreme of the protein, suggesting that CD39L2 has a short putative cytoplasmic tail and a large extracellular C-

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terminal domain (FIG. 5). There are two potential N-glycosylation sites in the predicted extracellular domain. A cAMP and cGMP-dependent protein kinase and a protein kinase-C phosphorylation site are found directly after the initiation
5 methionine codon (nucleotide 232).

Isolation and Sequence Characterization of CD39L3. An additional novel gene and polypeptide CD39-like sequence, referred to herein as CD39L3, was also identified. BLASTN search of the NCBI EST database with the full cDNA sequence for human CD39 (Accession No. S73813) identified three EST
10 entries for cDNA clones from an endometrial tumor library (Accession Nos. AA336644, AA338117, and AA337885), which led to the identification of an IMAGE library EST (Accession No. N72742) that was completely sequenced and had amino acid homology to CD39 and CD39L1. The insert of N72742 was used to screen the Jurkat, ZR75, and KER cDNA libraries, and a
15 single cDNA clone was isolated from the KER library and sequenced.

A 1669-bp ORF was identified within the cDNA insert. The nucleotide sequence (SEQ ID NO:3) and derived amino acid sequence (SEQ ID NO:4) of the cDNA insert, referred to herein as CD39L3, are shown in FIG. 6. The amino acid sequence was
20 revealed to contain ACRs I-IV, an ATG codon at position 83, and a single polyadenylation signal at position 2758. Hydrophobicity plots as described above predict two potential transmembrane segments at the N and C-terminal extremes of the protein (FIG. 5). There are seven potential extracellular N-glycosylation sites. A cAMP- and cGMP-
25 dependent protein kinase site and a protein kinase-C phosphorylation site are located at the C-terminal extreme of the protein.

Isolation and Sequence Characterization of CD39L4. An additional CD39-like gene and polypeptide sequence, referred to herein as CD39L4, was also identified. A TBLASTX search
30 of the NCBI EST database with the full cDNA sequence for the mNTPase was performed. A human EST clone was sequenced, and an ORF was identified extending to nucleotide 529 of 2260 nucleotides that contained ACR I only and an ATG codon at

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position 256. In the same reading frame, downstream of the stop codon at nucleotide 529, an ORF extending to nucleotide 1792 contained ACRs II, III, and IV. Further analysis of the nucleotide sequence revealed a putative intron with splice donor and acceptor sites that conform to the 5' gt...3' ag rule (Breathnach and Chambon, 1981, Ann Rev. Biochem. 50:349-383; splice donor CAGgtcacttatggagcctg at nucleotide position 470, acceptor ccatggacaaaatagGAC at position 710, exon sequence underlined). Further analysis of the sequence revealed that removal of the 251-bp putative intron would result in a contiguous ORF containing ACRs I-IV. The hypothesis that this sequence does indeed constitute an intron was only confirmed by isolation and sequencing of three additional cDNA clones (CD39LAJ1-3) from the Jurkat library, one of which contained the 251 bp.

The nucleotide sequence (SEQ ID NO:5) and derived amino acid sequence (SEQ ID NO:6), referred to herein as CD39L4, is depicted in FIG. 7. The sequence contained a poly(A)tail, but no consensus polyadenylation sequence (Proudfoot, 1991, New Biol. 3:851-854). This is also the case for mNTPase. Hydrophobicity plots as described above predicted a single transmembrane segment at the N-terminal extreme of the protein (Fig. 5). This is similar to the predicted topology of CD39L2 and different from that of CD39, Cd39L1, and CD39L3. There are three potential extracellular N-glycosylation sites.

FIG. 8 depicts an alignment of each of the above-described sequences, along with other members of the NTPase CD-39-like gene family. The ACR domains are indicated by arrows.

Expression of the Human Members of the CD39-Like Family.

Representative probes for each member of the CD39-like gene family were hybridized to human multiple tissue Northern blots. Results are presented below.

CD39L2. Hybridization of the CD39L2 cDNA insert to the multiple tissue Northern blots resulted in two prominent signals of 2.6 kb (major) and 4.4 kb (minor) in all tissues

studied. This is most likely due to differential polyadenylation.

CD39L3. A PCR product covering the coding sequence of CD39L3 was used for the Northern hybridizations. A strong signal of approximately 3.0 kb could be seen in adult brain, pancreas, spleen, and prostate. Though moderate or low expression was seen in most other tissues, no signal was detected in adult liver and peripheral blood leukocytes. A weaker signal of approximately 1.8 kb was found in adult pancreas and may be the result of alternative splicing.

CD39L4. The CD39L4 cDNA was hybridized to the same Northern blots, and a prominent signal of approximately 4.8 kb was seen in adult liver, kidney, prostate, testis, and colon. Considerably weaker expression was seen for all other tissues examined. Several smaller bands were observed in tissues showing the strongest expression of CD39L4 and may be the result of differential polyadenylation or alternative splicing.

Mapping of Members of the CD39-like Family.

The CD39L2 gene was mapped with a lod score of >19 to human chromosome 20 by PCR typing of the GeneBridge 4 Radiation Hybrid Mapping Panel (Gyapay et al., 1996, Human Mol. Genet. 5:339-346). CD39L2 mapped 9.76 cR from D20S493 (typing data: 12012 02101 22000 00111 00110 01210 00110 01101 10121 00100 00120 11211 00011 11012 01001 01102 00000 00000 001). Using the closest flanking markers (D20S184 and D20S99) also represented on the consensus map, this placed CD39L2 at chromosome band 20q11.2. The location of CD39L2 on human chromosome 20 was confirmed by PCR analysis of monochromosomal mapping panels (Kelsell et al., 1995, Ann. Human Genet. 59:233-241). On the basis of synteny to human chromosome 20q11.2, the mouse homolog of CD39L2 was expected to map to mouse chromosome 2 (DeBry and Seldin, 1996, Genomics 33:337-351).

The CD39L3 gene was mapped as described above to human chromosome 3, 5.76 cR from D3S3390 (data: 12002 02010 22000 00011 20000 00110 01001 00000 02022 11000 10001 00200 21100

plots as described above predicted a single transmembrane segment at the N-terminal extreme of the dCD39L4 protein. The topology of dCD39L4 is therefore most similar to the predicted topology of the CD39L2 and Cd39L4 proteins.

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The present invention is not to be limited in scope by the exemplified embodiments which are intended as illustrations of single aspects of the invention, and compositions and methods which are functionally equivalent are within the scope of the invention. Indeed, numerous
10 modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the present preferred embodiments. Consequently, the only limitations which should be placed upon the scope of the invention are those which appear in the appended claims.

15 All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.

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